REMARKS

This application has been amended in a manner that is believed to place it in condition for allowance at the time of the next Official Action.

Claims 1-20 are pending in the present application. Claims 1-17 have been amended to address the formal matters raised in the outstanding Official Action. Claims 18-20 have been added. Support for claims 18-20 may be found in original claim 1 and in the present specification. In particular, support for the new claims may be found at page 5, lines 25-30 and page 10, lines 1-10.

In the outstanding Official Action, claim 15 was objected to for reciting the term "comprises comprising". However, claim 15 has been amended to correct this informality. As a result, applicants respectfully request that the rejection be withdrawn.

In addition, claim 15 was rejected under 35 USC §112, second paragraph, for allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention. Applicants believe that the present amendment obviates this rejection.

In imposing the rejection, the Official Action alleged that the phrases "high initial cell concentration" and "which corresponds to a total duration of" were indefinite. However, claim 15 has been amended so that the term "high" has been

deleted. In addition, the phrase "which corresponds to a total duration of" has been deleted. As a result, applicants believe that claim 15 is definite to one of ordinary skill in the art.

Claims 1-16 were rejected under 35 USC §112, first paragraph, for allegedly failing to comply with the written description requirement. This rejection is respectfully traversed.

In imposing the rejection, the Official Action states that "the disclosure lacks an adequate written description for the materials required to practice the claimed invention. Specifically, the Official Action alleged that the embodiments of embryonic stem cells at the origin of somatic stem cells, stem cells at the origin of various solid tissues and the specific inhibitor of cell development were not adequately described by the instant specification." Applicants believe that the present amendment obviates the rejection.

In the amended set of claims all references to "stem cells" have been specified as "human stem cells", with the exception that new claims 19 and 20 recite primate stem cells. As a result, it is believed that the claimed invention complies with the written description requirement. Moreover, the use of TGFβ as a preferred inhibitor of cell development is described in detail. In regards to "human stem cells", they are exemplified by human hematopoietic stem cells.

Applicants submit that it would be within the ability of the skilled artisan to make minor adjustments to the experimental protocol set forth in the present application to other human stem cells and other inhibitors of cell development (or pleiotropic factors). Several papers demonstrate that the invention can be effectively implemented on various human stem cells and with various inhibitors of cell development as follows:

- the method and process of the invention have been implemented on human epidermal precursor cells or keratinocyte stem cells (which are an instance of "human stem cells" and more precisely of "human stem cells/somatic progenitors at the origin of blood and/or various tissues") by administering TGF-β1 as an inhibitor of cell development, as is explained in J. Cell Sci. 116:4043-4052. A copy of the article is attached herewith.

Thus, the skilled artisan who would read the disclosure of the present application would know which cells are within the scope of "human stem cells" and would know what the expression "inhibitor of cell development" more particularly refers to (examples of inhibitors of cell development are to be found on page 3, lines 10-29).

Moreover, the specification provides guidelines for how adjustments ought to be made so as to implement the method or process of the invention on other human stem cells than human hematopoeitic stem cells. For example, as explained on page 10, lines 21-25, these adjustments may consist in the choice of a

differentiation medium: "the differentiation medium [...] is chosen according to the tissue or organ it is intended to reconstitute. For example, if the intention is to obtain erythroid cells, a culture medium comprising erythropoietin (EPO) will be used".

Indeed, the choice of a specific differentiation medium so as to obtain cells of a specific tissue clearly belongs to the normal capacities of the skilled person.

Thus, in view of the above, applicants respectfully request that the written description rejection be withdrawn.

Claims 1-16 were rejected under 35 USC §112, first paragraph, for allegedly not satisfying the enablement requirement. This rejection is respectfully traversed.

The Official Action contends that "when taken with the lack of any particular and specific guidance provided by the specification for the specific factors, specific stem cells and specific conditions to culture the stem cells in, to achieve inhibition of differentiation, yet allow for cell division, the lack of working examples, the breadth of the claims, with regard to any particular stem cells and any particular factor, it would have required undue and unpredictable experimentation for one of skill in the art to practice the claimed invention."

However, the Examiner is respectfully reminded that it is a well founded principle that any assertion by the Patent Office that the enabling disclosure is not commensurate in scope

with the protection sought must be supported by evidence or reasoning substantiating the doubt so expressed.

As a matter of law, the expressed teaching of the patent specification cannot be controverted by mere speculation and unsupported assertions on the part of the Patent Office. As stated by the Court of Customs and Patent Appeals in the case of *In re Dinh-Nguyen and Stanhagen,* 181 USPQ 46 (CCPA 1974):

> Any assertion by the Patent Office that the enabling disclosure is not commensurate in scope with the protection sought must be supported by evidence or reasoning substantiating the doubt so expressed. 181 USPQ at 47.

Such a standard must be applied with great care when the Examiner's conjecture is contrary to the teachings of the specification.

Moreover, the Examiner's attention is respectfully drawn to the fact that the breadth of the claims has been restricted. Thus, the amount of experimentation required for one skilled in the art to practice the claimed invention has been considerably reduced and is now thought to comply with the enablement requirement.

As already discussed above, the specification provides clear and simple guidelines for the skilled person who would want to depart from the specifically illustrated human hematopoietic stem cells and TGFβ to work with other human stem cells. It is also believed that the level of unpredictability in the art underlined by the Examiner has been considerably reduced by

excluding from the scope of the invention stem cells from species other than human and primate. In support of this statement are the various instances of implementing the invention that have been made of record.

Furthermore, most of the populations of human stem cells that are different from the population of human hematopoietic progenitors described in the example of the present application are actually simpler for a skilled artisan to deal with than said population of hematopoietic progenitors. Indeed, the population of hematopoietic progenitors is a rather heterogeneous population, whereas populations of cells such as human embryonic stem cells or keratinocyte stem cells are more homogeneous and allow an implementation of the invention by a simple addition of TGFβ (see article J. Cell Sci. 116:4043-4052) instead of the more elaborate steps of successively culturing the cells with anti-TGFβ and TGFβ that are undertaken for implementing the invention on the system of hematopoietic progenitors (see example in present specification). Indeed, the present disclosure provides an elaborate example of implementing the invention; other implementations are therefore within the ability of the skilled artisan.

It is therefore believed that the skilled person is able to practice the claimed invention, additionally using his or her ordinary skill in the art, for instance for choosing a

specific differentiation medium adapted to the particular population of cells to be obtained.

In view of the above, applicants respectfully request that the enablement rejection be withdrawn.

In the outstanding Official Action, claims 1, 3-7, 8-10 12, 15 and 16 were rejected under 35 USC §102(b) as allegedly being anticipated by WILLIAMS et al. This rejection is respectfully traversed.

The Official Action alleges that "Williams teach the culturing of mouse ES cells in the presence of the leukemia inhibitory factor (LIF), which is a molecule that induces differentiation in myeloid leukemic cells. [...] William further teaches that long-term maintenance of the four ES cell lines in LIF for up to 22 passages (approximately 100 cell generations) did not alter the growth characteristics of the cells and they maintained the stem-cell phenotypes."

However, a major difference between the claimed invention after amendment of the claims and this prior art document is that cells in Williams et al. are of murine origin, whereas the cells of the invention are human or primate.

Indeed, as acknowledged by the Official Action, there exists a level of unpredictability in the art encountered when the skilled person wants to adapt a method which is efficient in a particular species to an unrelated species such as from primate to murine.

This level of unpredictability would not have allowed one with ordinary skill in the art to replace mouse embryonic stem cells with human or primate somatic stem cells. Indeed, LIF, which is used in Williams et al., has been shown to be ineffective for enabling self-renewal without differentiation of human embryonic stem cells (see article Stem cells 22:770-778 by Dahéron et al., attached herewith), whereas TGFβ is effective on such human embryonic stem cells (see present specification).

Claims 1-16 were rejected under 35 USC §102(b) as allegedly being anticipated by XI et al. This rejection is respectfully traversed.

In imposing the rejection, the Official Action states that "Xi compare the mechanisms of platelet factor 4 (PF4) and TGF-β1 on the growth of megakaryocyte (MK) progenitor cells in CD34+ cells. Xi teach that although both PF4 and TGF-β1 inhibit MK development from CD34+ cells, they show different effects in this inhibition. Where the inhibition of PF4 is found to be reversible, the inhibition using TGF-β1 is not."

However, XI et al. discloses that differentiation of CD34+ cells along the MK lineage is inhibited by TGF-β1 and PF-4. As stated on page 2 of the specification, this property of TGFβ was already known. XI et al. also compares the mechanisms of inhibition by these two factors.

However, the present invention also allows for the multiplication of stem cells while maintaining them in a non-

differentiated state. XI et al. does not teach anything about multiplying stem cells using an inhibitor of cell development in combination with an anti-inhibitor.

In all of the experiments conducted by XI et al. with TGFβ, an inhibitory effect on proliferation is shown, instead of a multiplying effect. See, for instance, Figures 3 and 4. Hence XI et al. does not teach how to multiply cells without differentiating them using TGFβ.

For example, the cells XI et al. focus on are later progenitors rather than the stem cells described in the example of the present application (e.g., CD34+ CD38- cells described on page 14). XI et al. only works with later megakaryocyte progenitors.

Moreover, in regards to the experiments conducted by XI et al. with PF4, it is said on page 265 that "PF4 does not seem to inhibit the proliferation of high-proliferative potential mixed colony-forming units-megakaryocytes". This is what a low concentration of TGFβ is able to achieve in a reversible manner. Indeed, single cell experiments have shown that anti-TGFβ can activate such HPP-mCFU-MKs (see Batard et al., J. Cell Sci. 113:383-390).

Thus, in view of the above, XI et al. does not disclose nor suggest the claimed invention.

Claims 1, 2, 4, 5-9, 12 and 14 were rejected under 35 USC §102(e) as being anticipated by MOORE et al. This rejection is respectfully traversed.

The Official Action alleges that "Moore [...] teach a protein, pylartin, which can be used in culturing hematopoietic stem cells of various species which allows the preservation of the progenitor cells, yet is able to inhibit the differentiation of the cells. They teach that the pylartin protein can be used in conjunction with flk2 ligand in an amount sufficient to selectively expand the progenitor cells, without inducing differentiation [...]." Applicants believe that the present amendment obviates this rejection.

Although MOORE et al. uses CD34+ cells in the various examples dealing with the effect of pylartin, MOORE et al. does not use culture conditions such as those used in the present application (i.e., TPO, FL, SF, IL6) to amplify the CD34+/CD38- subpopulation. Instead, IL3 is used in the control stem cell assay, and yet it is known that IL3 differentiates early progenitors.

MOORE et al. studies the effect of pylartin on CD34+ cells. However, the CD34+ cells used by MOORE et al. are not necessarily representative of the stem cell compartment. Indeed, the stem cell compartment represents only 1% of CD34+ cells. This is why MOORE et al. does not show a control or activation by pylartin on primitive quiescent hematopoietic stem cells.

Therefore, the claimed invention is novel over MOORE et al.

Claims 1-10 and 12 were rejected under 35 USC §103(a) as allegedly being unpatentable over HATZFELD et al. This rejection is respectfully traversed.

The Patent Office alleges that "Hatzfeld teach that TGFβ down-modulates various cytokine receptors, and that this effect can be suppressed within 6 hours by the addition of anti-TGFβ antibodies or antisense nucleotides. Hatzfeld study the release from TGFβ growth inhibition of HPPQ primitive progenitors to understand whether this inhibitor is a cental regulator of the stem cell compartment. They teach that these observations are used in developing an in vitro assay which combines receptor induction by anti-TGFβ together wit optimal cytokine stimulation which can be performed using non-purified hematopoietic progenitors. They teach that this method can render quiescent primitive progenitors responsive to optimal combinations of cytokines to improve the in vitro expansion of clinical samples. Thus they teach the neutralization of an inhibitor of cell development (i.e. TGFβ). Although Hatzfeld do not specifically teach that the cell divisions range from 1 to 11 [...] it would be obvious for one of ordinary skill that by allowing the cells to divide there would be at least one cell division that occurs."

The abstract cited by the Patent Office primarily focuses on the effect of TGFβ and anti-TGFβ on various receptors,

and more particularly pertains to the use of anti-TGFβ for rendering quiescent hematopoietic progenitors sensitive to cytokine stimulation.

Yet, this publication does not disclose nor suggest how human stem cells can be multiplied in vitro while being maintained in a non-differentiated state. In particular, the abstract does not disclose nor suggest the beneficial effect of adding an inhibitor of cell development such as TGFβ for maintaining a "stem" state during cell divisions. Neither does it disclose nor suggest how to use TGFβ and anti-TGFβ in a sequence combination or cyclically. Indeed, it is believed that the skilled artisan would be deterred by the abstract from using TGFβ or anti-TGFβ to multiply non-differentiated stem cells, since the abstract concludes by mentioning the possibility of using "transient activation of HPPQ" as "an excellent tool to mark stem cells and follow their development", which suggests pushing the cells towards further differentiation instead of maintaining them in a non-differentiated state. Thus, applicants believe that the publication actually teaches away from the claimed invention.

Thus, in view of the above, applicants believe that Hatzfeld fails to render obvious the claimed invention.

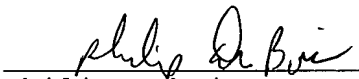
In view of the present amendment and the foregoing remarks, therefore, applicants believe that the present application is in condition for allowance, with claims 1-20 as

presented. Allowance and passage to issue on that basis is respectfully requested.

The Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 25-0120 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17.

Respectfully submitted,

YOUNG & THOMPSON



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APPENDIX:

The Appendix includes the following items:

- "LIF/STAT3 Signaling Fails to Maintain Self-Renewal of Human Embryonic Stem Cells" by L. Dahéron et al., Stem Cells 22:770-778;

- "Long-term expansion of human functional epidermal precursor cells; promotion of extensive amplification by low TGF-β1 concentrations" by N. Fortunel et al., J. Cell Sci., 116:4043-4052;

- "TGF-β1 maintains hematopoietic immaturity by a reversible negative control of cell cycle and induces CD34 antigen up-modulation" by P. Batard et al., J. Cell Sci., 113:383-390.